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Short Communication

Growth of eye lens weight and age estimation in the northern red-backed vole, *Clethrionomys rutilus*

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Age determination is a basic requirement when analyzing the ecological events affecting wild animals. Several useful methods for age determination have been reported for small rodents (see Pucek and Lowe 1975). Tooth wear patterns and the molar root ratio have often been used to assess the ages of Japanese rodents (Abe 1976, Fujimaki *et al.* 1976, Fujimaki 1977, Hikida and Murakami 1980). Furthermore, since Lord (1959) proposed a method of age determination using the eye lens weight (ELW) in cottontail rabbits, *Lepus americanus*, it has become well-known that ELW can also be employed as an age criterion in various species of Rodentia (Östbye and Semb-Johansson 1970, Gourley and Jannett 1975, Hagen *et al.* 1980, Thomas and Bellis 1980, Ando and Shiraishi 1997 for the subfamily Microtinae, and Berry and Truslove 1968, Adamczewska-Andrzejewska 1973, Yabe 1979, Okamoto 1980, Takada 1982, Hardy *et al.* 1983, Takada 1996 for the subfamily Murinae). It is considered that the ELW method of age estimation is more accurate than any other technique relying on body or skull measurements (Pucek and Lowe 1975). Moreover, this method has the advantage that it can be used for species which have rootless molars such as *Eothenomys smithii* (Ando and Shiraishi 1997).

The growth of the molar roots of the northern red-backed vole, *Clethrionomys rutilus* was examined as an indicator of absolute age by Tupikova *et al.* (1968), and the relationship between lens weight and age was analyzed using specimens captured in the field (Askaner and Hansson 1967), however, no previous data on the growth patterns of eye lens from known-age individuals have been reported for this species.

An accurate method for age determination is of value not only for ecological studies of *C. rutilus* itself, but also for analysis of the transmission pattern of a zoonosis in a natural population. The latter is of particular significance because *C. rutilus* is one of the favorable intermediate hosts of *Echinococcus multilocularis*, a parasitic organism causing the serious disease alveolar echinococcosis in humans, which has been found in Hokkaido, Japan (Takahashi and Nakata 1995).

The purpose of the study described here, therefore, was to establish an age estimation equation by analyzing the relationship between the growth in eye lens weight and age in a population of known-age laboratory-reared northern red-backed voles.

Materials and Methods

A total of 197 voles (91 males and 106 females) from a laboratory colony originating from wild voles captured in Sapporo, Hokkaido were used in this study. The laboratory colony was maintained under regulated conditions at a temperature of 23–25°C a 12 hour light and 12 hour dark photoperiod and fed a commercial diet (CMF, Oriental Yeast Co. Ltd.). Voles were reared individually in mouse cages except for during breeding. Male voles ranged in age from 15 to 596 days, and females from 15 to 581 days. Voles were killed with ethyl ether, their eyes were dissected out and fixed in 10% formalin at room temperature for at least four weeks, then the optic lenses were excised and washed with distilled water. Lenses were dried in an oven at 80°C for 24 hours and immediately weighed to the nearest 0.01 mg on a microbalance (Mettler, AT201).

Results and Discussion

Lens weight was found to increase rapidly up to about day 50 and then the growth rate decreased gradually in both males and females (Fig. 1), as has also been noted for *Lemmus lemmus* (Östbye and Semb-Johansson 1970). In this study, ages were selected non-randomly and measured without error. For this reason, in the regression analysis of this data, age is the independent variable and lens weight the dependent variable with lens weight regressed on age (Hagen *et al.* 1980). Ages were logarithmically transformed, because the growth pattern of lens weight was found to be curvilinear in *C. rutilus* (Fig. 1).

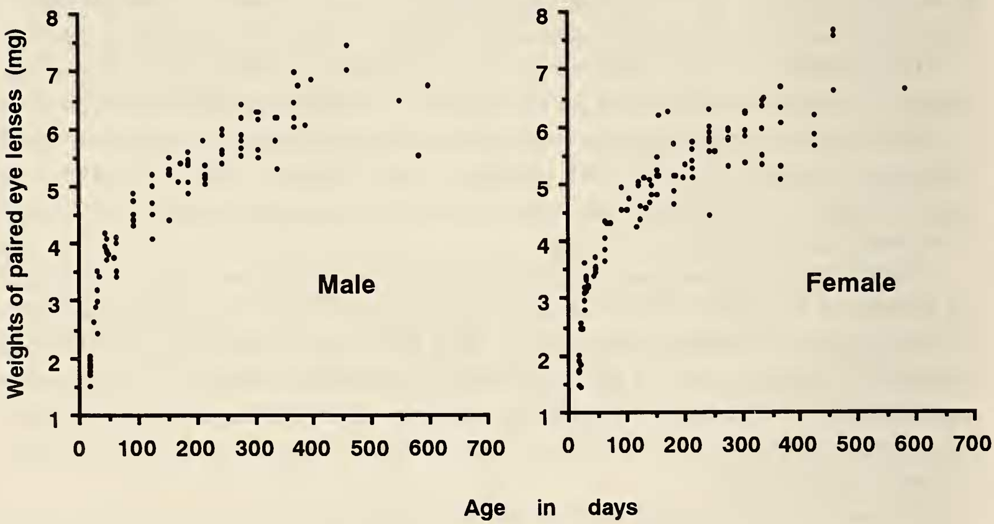

Fig. 1 Growth of the eye lens weight in 91 male and 106 female northern red-backed voles, *Clethrionomys rutilus*.

Fig. 2 Relationship between log-transformed ages and eye lens weight in the northern red-backed vole, *Clethrionomys rutilus*.

Moreover, array variance must be of equal magnitude along the length of the line (homoscedasticity) in the regression analysis (Dapson 1980), and homoscedasticity was confirmed with the graphs showing residuals plotted against Y_i (the Y value on the line X_i) for both sexes. The simple linear regression relationship ($Y=a+bX$) between X (age in days after logarithmic transformation) and Y (lens weight) was applied (Fig. 2). Regression equations from our data from *C. rutilus* were:

(1) $Y=-1.433+3.000X$ ($r^2=0.935$, $p<0.001$) for males
(2) $Y=-1.322+2.939X$ ($r^2=0.908$, $p<0.001$) for females

where Y=weight of paired lenses in mg, $X=\log_{10}x$, and x=age in days.

There were no significant differences in regression slopes between males and females (F-test, $F_{cal}=0.24$, $F_{0.05(1,193)}=3.89$, $p>0.05$) (Table 1). Age was predicted inversely from either equation (1) or (2), and predicted age was given by the equation:

$\hat{x}=10^{(Y+1.433)/3.000}$ for males
$\hat{x}=10^{(Y+1.322)/2.939}$ for females

The equation of the 95% confidence limits (L) for the inverse prediction is given as follows:

Table 1. Statistics on the regression lines between the age and eye lens weight in the northern red-backed vole, *Clethrionomys rutilus*.

Sex	n	a	b	r	$\bar{X}$	$\bar{Y}$	S_{YX}	SS_X	t
Male	91	-1.433	3.000	0.967	2.064	4.760	0.382	20.638	1.987
Female	106	-1.322	2.939	0.953	2.061	4.736	0.426	21.630	1.983

n: number of samples, a: Y intercept, b: slope, r: correlation coefficient, $\bar{X}$: mean of X, $\bar{Y}$: mean of Y, S_{YX}: standard error of estimate, SS_X: sum of squared deviations of X, t: Student's t ($d.f.=n-2$, $p=0.05$).

$$L=\bar{X}+\frac{b(Y_i-\bar{Y})}{K}\pm\frac{t}{K}\sqrt{S^2{}_{YX}\left[\frac{(Y_i-\bar{Y})^2}{SS_X}+K\left(\frac{1}{m}+\frac{1}{n}\right)\right]}$$

where $\bar{X}$ = the mean of X, $\bar{Y}$ = the mean of Y, $K=b^2-t^2S_b{}^2$, t = Student's t ($d.f.=n-2$, $p=0.05$), S_b = the standard error of the regression coefficient, $S^2{}_{YX}$ = the residual mean square, SS_X = the sum of squared deviations of X, n = sample size, and m = the number of individuals upon which predictions will be based. Here, when $m=\infty$, L indicates the confidence limits of the mean prediction for the population. On the other hand, when $m=1$, L indicates the confidence limits of the individual prediction (Dapson 1980, Sokal and Rohlf 1981).

When estimating the ages of individual animals, Dapson (1980) pointed out the importance of presenting the 95% confidence limits, as the confidence interval indicates the accuracy of an estimate of age for each specimen, and the confidence interval for the individual prediction is generally broader than that for the population. This certainly proved to be the case in *C. rutilus* (Tables 2 and 3). In this study, broader ranges in the 95% confidence interval were observed among older animals because of the wide variance of lens weight and the decrease in the growth rate in these older animals (Fig. 1).

Askaner and Hansson (1967) examined the relation between ELW and molar root length of wild-caught *C. rutilus*, and pointed out the usefulness of the ELW method for aging individuals of this species. The present study provides, for the first time, an equation for age estimation based on ELWs of known-age voles. Tupikova *et al.* (1968) developed an age determination

Table 2. Predicted ages and confidence limits (95 %) for the mean and individual predictions at given lens weights in the male northern red-backed vole, *Clethrionomys rutilus*.

Lens weight (mg)	Predicted age in days	Mean predictions		Individual predictions	
		Lower age limit	Upper age limit	Lower age limit	Upper age limit
1.5	9	8	11	5	17
2.5	20	18	23	11	37
3.5	44	40	48	24	79
4.5	95	89	101	53	171
5.5	205	191	219	114	369
6.5	442	402	487	245	799

Table 3. Predicted ages and confidence limits (95 %) for the mean and individual predictions at given lens weights in the female northern red-backed vole, *Clethrionomys rutilus*.

Lens weight (mg)	Predicted age in days	Mean predictions		Individual predictions	
		Lower age limit	Upper age limit	Lower age limit	Upper age limit
1.5	9	7	10	4	18
2.5	19	17	22	10	38
3.5	43	39	47	22	83
4.5	94	88	100	48	182
5.5	205	191	221	105	400
6.5	451	406	502	230	883

method for *C. rutilus* using the length of the root and the height of the crown of M^2, however, since the neck of M^2 in this species is not formed until three months old, the ages of young voles under two months old cannot be predicted by this method. The present results show that the ELW technique is capable of estimating age in this species, especially in younger voles. For application of this technique to field studies, however, we must pay attention to the wide confidence interval in older voles.

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